On the Strobilization of Aurelia.

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With Plate 6 and 3 Text-figures.

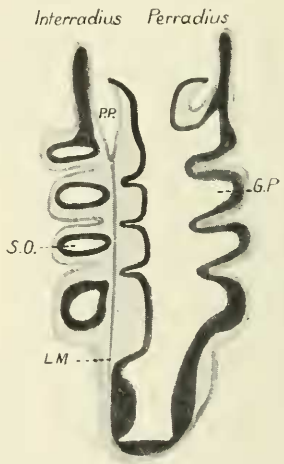
1. Introductory and Historical.

It is well known that in the production of a polydisc strobila the scyphistoma undergoes a series of constrictions as far as the septal longitudinal muscle-strands, commencing just below the wreath of tentacles and continuing downwards towards the foot. There is always a portion at the pedal end which does not undergo constriction, remaining ultimately as a polyp after regeneration of oral disc and tentacles. The animal at this stage is a pile of segments, the oldest and most defined being at the oral end, while the youngest and least defined is near the pedal end. Later, there usually arise from each segment, or ephyra rudiment, eight radiating lobes enclosing diverticula from the enteron, four in the interradii and four in the perradii. At the end of each lobe develops a median outgrowth containing an extension of the enteron, which becomes the tentaculocyst. On each side of the tentaculocyst is a wing-like lappet of ectoderm.

The scyphistoma, just before undergoing the external changes, has four radiating septa perforated each by an ostium beneath the oral disc, forming together a 'gastral ring-sinus'. As the body increases in length new ostia arise below the old ones (Text-fig. 1), and the constriction takes place between the sets of perforations which later take part in the formation of the gastral cavity of the ephyra.

There appears to be unanimity of opinion between previous workers on this portion of the change, but with regard to later developments there is some diversity of view. Perhaps they are least unanimous on the subject of the origin of the manubrium of the non-terminal ephyra. 'According to Goette it proceeds from a completely new formation after the casting off of the preceding ephyra' (Heric). Claus (1) figures Aurelia as having, between two ephyra rudiments, a circular shelf or horizontal fold of the two-layered wall of the connecting tube. The ectodermal portion of the fold is apparently continuous all round, but the endodermal portion is interrupted in

TEXT-FIG. 1.



Early stage in strobilization.

each interradius. Its four radial components appear from within as grooves, the adjacent ends of which grow towards each other and meet between the longitudinal muscle-strand and the ectoderm, thus forming the continuous ring-canal which Claus calls the 'ring-sinus of the proboscis (i.e. manubrial) disc'. As a result of this the septal or longitudinal muscle-strand in the region connecting adjacent ephyrae is surrounded by endoderm, and when the edge of the incipient manubrium becomes split off from the exumbrella of the preceding ephyra, it bends outwards, leaving the septal muscles to act as connecting strands between the two ephyrae. In fact the original neck connecting one ephyra with its neighbour is now reduced to the four longitudinal muscle-strands, each surrounded by a strip of endoderm, while the wall of the neck has become converted into the manubrium and spread out horizontally.

Heric (**4**), working on Chrysaora, shows the intersegmental folds, but states that they occur only between the septa as four bladder-like outgrowths of both layers. These, on breaking away distally from the exumbrella of the upper ephyra, spread outwards as four semicircular flaps the adjacent edges of which meet and fuse in the interradii, so producing the flat manubrium. Thus, he says, the connecting strands have endoderm on the inner side and ectoderm on the outside (whereas in Aurelia, according to Claus as we have just seen, ectoderm is entirely absent).

Claus and Heric show, then, that the manubrium is undergoing development at the same time as the rest of the ephyra, and that the lining of the manubrium is of endoderm.

Claus also states that the manubrium of the polyp, which remains after strobilization, is lined by endoderm, so that at no time is there a stomodaeum such as is described by Goette. The work of Friedemann (**3**) and Hein (**2**) on the embryological aspect confirms the above as regards the endodermal lining of the manubrium and the absence of stomodaeum.

Claus and Heric state that the gastral filament is derived by the transformation of the columella, i. e. the axial portion (internal to the ostium) of the taeniole containing the longitudinal muscle-strand, which for a time connects the subumbrella and exumbrella of a developing ephyra.

With regard to the septal muscle-strands Goette has described them as hollow structures with the cavity continuous with that of the peristomial pit. Claus (**1**), Friedemann (**3**), and Heric (**4**) state that the muscles are solid structures, and Friedemann figures the peristomial pit of a well-developed scyphistoma as being distinct from the septal muscle.

There does not appear to be any record of an observation on the development of the peristomial pits in a non-terminal ephyra. Claus (loc. cit.), in one figure, indicates a peristomial pit on one non-terminal ephyra but shows nothing further. Heric concludes that they are new structures.

In the same way there is little information on the regeneration of the oral disc of the polyp which remains after strobilization. Heric indicates that the development of the proboscis is similar to that of the manubrium of the ephyra, but says that the four strands connecting the polyp with the ephyra above pass directly to the wall of the enteron after having become dissociated from the proboscis. According to this the polyp would have no septal ostia, and the oral end of the longitudinal muscle would be absent for a time. Whether this is so or not in Chrysaora I cannot say, but, as shown below, it is not the case in Aurelia. He goes on to say: 'How the oral disc (of the polyp) is connected with the septa can be answered just as little as the question whether the septal muscle, in its course, is transformed by the remainder of the polyp to be replaced by a new muscle originating from the oral disc, or whether it is preserved and enters (secondarily) into connexion with the oral disc'.

A complete ephyra becomes free by the gradual extension of the connecting strands and by their final rupture. Prior to this event the exumbral opening has closed. Heric believes that this closure takes place simultaneously with the breaking of the strands. He did not find any trace of longitudinal muscle in an ephyra immediately after detachment.

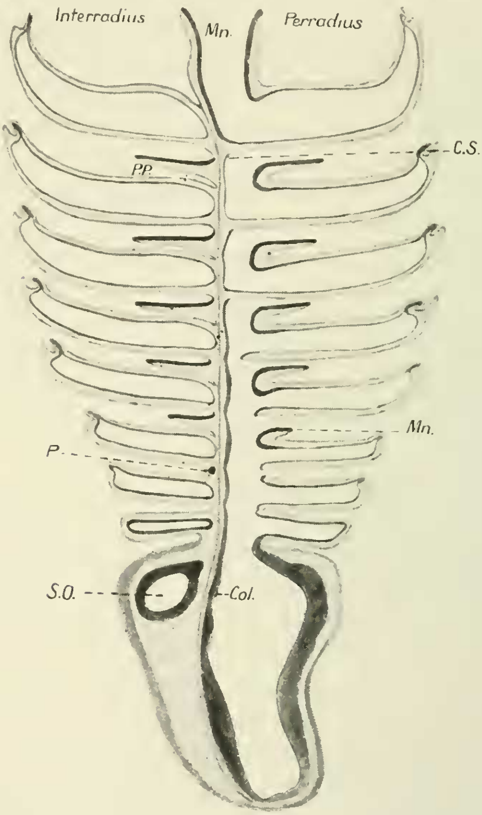
2. New Investigation.

The material used in this work was obtained from the Dove Marine Laboratory, Cullercoats, Northumberland, and was killed and fixed in saturated aqueous corrosive sublimate solution with 2 per cent. glacial acetic acid. Delafield's haematoxylin, and iron haematoxylin and eosin were used to stain the serial sections. Delafield's haematoxylin is very useful for general observations, but the latter stains were better for determining the limits of ectoderm and endoderm, especially in the early stages of the manubrium.

It should be understood that my description of the formation of an ephyra does not refer to the transformation of the original oral disc of a scyphistoma, but to the fate of the segments formed below the original oral disc.

Formation of Manubrium.—The constriction of a polyp proceeds as far as the longitudinal muscle, the ectodermal groove interradially cutting the septum horizontally. Between the interradii the endoderm is pushed inwards, but the edge so formed does not reach the imaginary line taken between two adjacent longitudinal muscles. This edge is concave internally, so that a transverse section between two ephyra rudiments would show a roughly cross-shaped gastral cavity with the longer diameters lying in the perradii. About this time a groove appears in the subumbral endoderm near the inner edge, beginning in the perradius as a very shallow groove (Pl. 6, fig. 12 *a*) and becoming deeper passes towards the septum or taeniole. The fold of endoderm thus formed pushes into the mesogloea between the subumbral endoderm and ectoderm (Pl. 6, fig. 12 *b*, *Gr.*). The groove, as it approaches the taeniole, divides into two portions at its septal end. One of these portions is a gutter passing round on the inside of the septal muscle to meet its fellow from the other side, and the other portion is a solid plug of cells (Pl. 6, fig. 12, *P.*) which grows through the septum towards the interradius, where it meets and fuses with a fellow from the other side, lying between the septal muscle and the ectoderm. The muscle is thus surrounded, in this manubrial region, by endoderm. The portion of the plug of endoderm cells lying in the interradius causes a bulging of the ectoderm at that point (Pl. 6, fig. 9), but the outer layer does not project all the way round. Four horizontal perradial slits now appear in the connecting tube in the same plane as the ectodermal projections (Text-fig. 2), so that the gastral cavity comes into communication with the outside. Finally, the slits unite across the interradial ectodermal projections and the outer layer of an upper ephyra is cut off from that of the next below. While this takes place, a split occurs in the plug of endoderm cells connecting the two endoderm grooves (Pl. 6, fig. 9, *S.*), which now become continuous abaxially to the longitudinal muscle. The lip of what is now the rudimentary manubrium, curls outwards involving the subumbral wall of the endodermal groove. Perradially, where

Text-fig. 2.



Determination of number of ephyrae and laying down of form of polyp.

there is practically no groove (Pl. 6, fig. 12 *a*) the curling causes a considerable increase in the diameter of the manubrial opening. This, no doubt, has some influence on the formation of the cruciate cavity of the manubrium. As Claus and Heric have shown, a manubrium formed this way has a lining of endoderm. It is, for a time, a flattened plate having a central opening into the enteron. This change does not involve the appearance of a 'proboscis ring-sinus' as described and figured by Claus, but it is possible that the solid rod of endoderm cells and the ring-sinus may be developed under different conditions.

Peristomial Pit.—Associated with the development of the manubrium is the formation of the peristomial pits. As has been stated, the constriction in the interradius brings the ectoderm very close to the longitudinal strand (they are separated by a thin layer of mesogloea), and when the endodermal plug pushes out the ectoderm a small interradial funnel is formed in the angle made by the fold and subumbrella (Pl. 6, fig. 9, *P.P.*); this is the rudiment of the peristomial pit. The subsequent curling outwards and growth of the manubrium and the increase in depth of the ephyra bring about the deepening of the funnel (Pl. 6, fig. 10, *P.P.*).

Longitudinal Muscle.—Contrary to what Claus, Friedemann, and Heric have maintained, transverse sections of a scyphistoma show that the septal muscles may be hollow structures (Pl. 6, fig. 8). The cavity may not be continuous along the whole length, but it generally appears a short distance below the peristomial pit, and, as Friedemann has shown, the end of the muscle and the apex of the pit are not continuous with each other. The muscle-cells are arranged with their tails to the mesogloea and the protoplasmic parts towards and projecting into the lumen (Pl. 6, fig. 8). There are mesogloeal ridges projecting into the cavity and on these are set muscle-cells, and this probably serves to increase the amount of muscular surface. Sometimes the cavity is quite wide, and none would describe such a structure as a solid muscle. Towards the foot the muscle-cells are usually closely packed, and this portion of the strand can be described as solid.

In a strobila, mainly in the lower segments, sections show that some parts of the muscles may be hollow, though the cavities in such cases are short, extending along the depth of an ephyra rudiment. As an ephyra becomes more developed and the circular and radial muscles begin to function, the longitudinal muscles begin to atrophy and are seen in longitudinal sections as mesogloeal bands striated with degenerate muscle-fibres (Pl. 6, fig. 11, *R.L.M.*). The bands stain more deeply than the rest of the mesogloea when treated with Delafield's haematoxylin.

Gastral Filaments.—About the time when the manubrium of the ephyra has become curled outwards the gastral filaments first appear. They occur in pairs, one pair per interradius (Pl. 6, figs. 1 and 5, *G.F.*). On each side of the columella, and close to the exumbral endoderm, there grows laterally and towards the central stomach an endodermal process (Pl. 6, fig. 1, *G.F.*). Very soon the tip turns towards the oral opening and remains pointing in that direction until the ephyra becomes free (Pl. 6, fig. 5). Occasionally one or both filaments may be suppressed. Thus, contrary to what has been maintained by all writers hitherto, the longitudinal muscle takes no part in the formation of the filaments which may be seen in a strobila in various stages of development.

Separation of an Ephyra from the Strobila.—In passing upwards towards the oldest ephyra the connecting strands are seen to become slightly stretched and the covering epithelium does not stain so deeply as that lower down. They converge to the apex of the exumbrella when the apical opening has become obliterated. The closure of this opening takes place comparatively early in the history of the attached ephyra (Text-fig. 2), and a free disc having such an opening may be considered to have been prematurely detached. Such may happen in the laboratory when a strobila is roughly handled.

Shortly before an ephyra becomes separated it is seen that the peristomial pits are very deep (Pl. 6, fig. 10, *P.P.*), and the depth from the subumbrella to the exumbrella is much greater than that in a younger disc. Also the columella is stretched so that the gastral filaments are brought some distance away from the exumbrella (Pl. 6, fig. 5). The covering of this stretched portion resembles in staining properties and cell-form that of the connecting strand in the same condition. The longitudinal muscle here is degenerate, and the apical portion of the ephyra is thickened with mesogloea in which the muscle remnants can be seen (fig. 5, *L.M.*). As Heric suggests, the separation is probably brought about by the violent action of the circular and radial muscle-bands. The connecting strands break close to the exumbrella. About the same time the stretched part of the columella breaks, resulting in the carriage of the gastral filaments to the subumbral side. The remnant of the stretched portion is seen as a small papilla of cells between the bases of the gastral filaments (Pl. 6, fig. 3, *R.C.*).

The rupture of the columella brings about a rapid shortening of the peristomial pit, which is now seen to be a small but distinct funnel-shaped depression (fig. 3, *P.P.*) in the subumbrella close to the bases of the gastral filaments. The greater part of the wall of the pit has gone to form a portion of the subumbral surface and the base of the manubrium. The pit, which was close to the base of the manubrium, is now some distance away. The gastral filaments no longer point to the oral opening but come to be roughly in a line at right angles to the interradius.

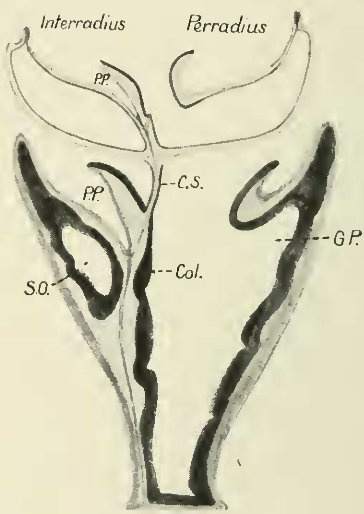
When an ephyra becomes free the connecting strands are left projecting from the oral opening of the next ephyra (Pl. 6, fig. 10, *C.S.*) and are soon reduced in length. The flat manubrium of this next disc now begins to assume the tubular form, which is not completed until separation and the rupture of the columella (Pl. 6, fig. 5).

Muscle remnants are to be found in a newly separated ephyra in the thickened apex (Pl. 6, fig. 4) and at the base of the manubrium on the inner side of the peristomial pit. A vestige of the connecting strand and the part of the muscle between it and the apex of the pit can be found at the base of the manubrium (Pl. 6, fig. 3, *R.L.M.*).

Formation of the Proboscis of the Polyp.—A strobila can be divided into two regions, viz. segmented, giving rise to ephyrae, and unsegmented, producing the polyp. Between every two adjacent constrictions lie four septal ostia arranged in a ‘ring-sinus’. Below the lowest constriction are four ostia which usually remain to form the gastral ring-sinus of the scyphistoma.

The proboscis of the polyp develops in a manner similar to that of an ephyra, but there is evidence of the formation of a proboscis ring-sinus such as Claus has described for the ephyra. The edge of the proboscis curls outwards but the opening is much wider than that in an ephyra. The passage of the

TEXT-FIG. 3.



Before liberation of last ephyra. Polyp complete.

connecting strands direct to the gastral wall of the enteron and unconnected with the wall of the oral opening was seen in only one case. Such a condition (which it will be remembered Heric regards as normal in Chrysaora) is probably accidental, and may be described in Aurelia as abnormal. In a case of this kind there are no septal ostia and so no gastral ring-sinus. The upper portion of the enteron is as entire as in the free ephyra. Usually the columellae persist and the resulting polyp possesses all the features of the original scyphistoma from which it has been formed (Text-fig. 3).

As far as has been ascertained the peristomial pit of the polyp does not arise in exactly the same manner as in the ephyra. It occurs in the same relative position but apparently not as a pit. A specimen showing the earliest trace had a solid strand of cells reaching from the oral disc obliquely inwards and downwards to the longitudinal muscle (Pl. 6, fig. 2, *R.*). At the outer end was a slight depression which was probably the commencement of the formation of a cavity in the strand. Another specimen showed a complete pit the apex of which bore the same relation to the longitudinal muscle as does that of an ephyra (see Text-fig. 3). Probably, then, the strand of cells becomes hollow from outside inwards. This cavity should not be confused with that in the longitudinal muscle, nor do the two ever communicate.

Ciliation of the Ectoderm.—Gemmill (**5**) has shown that, in the ephyra, there are definite currents passing over the ectodermal surface apparently for the purpose of carrying small animals to the lappets, where they are pierced by stinging threads and afterwards carried to the mouth by flexure of the arm. He also states that the scyphistoma captures infusoria in much the same way as the ephyra, the tentacles taking the place of the arm lappets.

Powdered carmine suspended in sea-water will also serve admirably to demonstrate these currents. In the scyphistoma the carmine particles are carried upwards along the surface of the body and become entangled in slime secreted by the ectoderm. Between the bases of the tentacles ropes of particles and slime may be seen carried along the disc and up to the edge of the proboscis. The tentacles, along which the current passes to the tip, sometimes curl over into the proboscis and the material travels into the gastric cavity. Often a slowly revolving ball is formed above the mouth and finally passes slowly down into the enteron. The same applies to the ephyra, in which the passage of the carmine ball into the gastric cavity can be easily seen. Sometimes the ball will be slowly ejected from the enteron immediately after being taken. Gemmill believes that particles are taken in ‘ by a central inhalant current which is compensatory to exhalant currents produced by ciliary action in the floor of the mouth angles ', adding ' but this may not be the whole explanation '.

In both ephyra and scyphistoma the use of powdered carmine seems to show that the lining of the proboscis or the manubrium has cilia which may produce exhalant or inhalant currents when necessary. The stream of particles passes down the angles in the perradii as well as along the ridges of the interradii; further, expulsion of material may follow the same channels. In only one case did I observe both currents acting simultaneously, and then the in-currents moved along the interradial ridge and the ex-currents along the perradial angles. A strobila shows a strong current from the foot upwards to the uppermost ephyra. This is due to the fact that the surface is composed of a considerable portion of the aboral surface of each ephyra, on which surface the current is centrifugal to the ends of the lappets. There did not appear to be any passage of carmine in between any two ephyra rudiments. This suggests that the intermediate ephyrae or ephyra rudiments do not feed by means of these currents.

High power examination shows that the currents are caused by flagellated ectoderm cells (Pl. 6, figs. 6 and 7). The ectoderm of the scyphistoma, except on the pedal disc, and of the ephyra appears to consist entirely of flagellated cells. Even the surface of the tentaculocyst is provided with flagella. When death takes place, either naturally or by poison, the flagella usually disappear. Occasionally in a well-fixed specimen they still persist and can be studied by means of sections. In the case of the lining of the manubrium or of the proboscis the flagella are almost always visible in sections, an interesting point which corroborates the histological evidence as to the endodermal nature of this lining.

Remarkable cases of Polyp Formation.—While keeping under observation a young strobila for the purpose of watching the transformation of the oral disc into an ephyra, I was able to observe a number of interesting but unexpected changes. There were five constrictions in the strobila, and the two ephyra rudiments next below the oral disc proceeded normally to give rise to ephyrae. The two rudiments below these, however, produced eight tentacles each and no lobes. The oral disc did not undergo any change except that it became separated from the rest and lay on the bottom of the dish. There was a large apical opening the presence of which suggested that the disc had been prematurely separated. After some days the hole closed and the tentacles were absorbed. No lobes developed and later a pedal stalk grew out from the apical end, the body ultimately becoming attached to the floor of the dish. Later four perradial tentacles appeared and the whole had the form of a normal scyphistoma. The next two rudiments proceeded normally and produced ephyrae which were subsequently liberated.

The rudiments possessing tentacles then became free and moved over the surface of the dish for about a week by means of the flagellated surface, one of them revolving, the other progressing in a more or less straight line. Later each grew a foot stalk from the apex and became attached to the vessel. In the meantime one had increased the number of tentacles to twelve.

A second young strobila also behaved in an unusual manner. Here the two segments next to the oral disc gave rise each to four tentacles and no lobes. They, along with the oral disc, became separated in a body and remained attached to each other while on the floor of the dish. The oral disc did not appear to undergo any change, but the other two segments gradually absorbed their tentacles and became converted into a long narrow stalk at the end of which was a pedal disc. Attachment with the dish was effected, the stalk became stouter, and the whole body assumed the form of a normal scyphistoma.

The occurrence of segments which give rise to polyps leads one to believe that there is a distinct difference between these and the ephyra rudiments. It may be that they have been derived from ephyra rudiments, the sequence of changes necessary to produce an ephyra having been interrupted or reversed, or they may be different from the start. Again, it may be that up to a certain point the segment may be regarded as undifferentiated and, in the cases where polyps are produced, they have remained so. It is interesting to note how they agree in general behaviour with the upper part of the base of the strobila, which produces oral disc and tentacles, while the segment above gives rise to an ephyra.

The several ways in which a polyp may be formed are thus :

By direct development from the egg.

By the outgrowth of stolons from a polyp.

By the elaboration of the basal portion of a strobila.

By the separation of an unchanged oral disc.

By the elaboration of an intermediate segment.

I wish here to acknowledge my indebtedness to Professor W. Garstang for the kindly criticism and help which, from time to time, he has afforded.

Summary.

The manubrium of a primarily non-terminal ephyra is formed from the connecting tube between two ephyra rudiments.

The proboscis of the polyp remaining arises in a manner similar to that of the manubrium of the non-terminal ephyra.

The apical opening of an ephyra normally closes before liberation.

The connecting strands are covered with endoderm.

The longitudinal muscles of a polyp may be hollow and the cavities are not in communication with the peristomial pits.

The formation of the peristomial pit is associated with the development of the manubrium.

The gastral filaments are formed as paired outgrowths of the endoderm of the columella. They do not involve the longitudinal muscle in their production. They originate early in the history of an ephyra.

There are definite currents over the ectoderm caused by flagellated ectoderm cells.

The segments of a strobila may give rise to ephyrae or polyps and the oral disc may separate unchanged to continue its existence as a polyp.

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EXPLANATION OF PLATE 6.

Fig. 1.—Longitudinal section of polydisc strobila showing relation between gastral filaments and columella.

Fig. 2.—Tangential section of base of strobila showing portion of solid rod of ectoderm cells passing from oral surface to longitudinal muscle.

Fig. 3.—Vertical section through interradius of newly liberated ephyra showing base of manubrium, peristomial pit, common base of gastral filaments, and remnant of longitudinal muscle.

Fig. 4.—Vertical section through apex of newly separated ephyra showing mesogloeal thickening and muscle remnants.

Fig. 5.—Median vertical section through uppermost ephyra of polydisc strobila.

Fig. 6.—Flagellated cell from ectoderm of scyphistoma.

Fig. 7.—Portion of living tentacle (*a*) extended, (*b*) contracted, showing flagella and cells.

Fig. 8.—Transverse section through connecting strand about half-way along a polydisc strobila, showing cavity of muscle, and muscle fibres.

Fig. 9.—Longitudinal section (somewhat oblique) cutting interradius of two lowermost ephyra rudiments showing origin of manubrium.

Fig. 10.—Longitudinal section (oblique) through interradius of strobila showing relation between peristomial pit and longitudinal muscle of second ephyra down.

Fig. 11.—Longitudinal section through centre of third ephyra down showing closure of apical opening.

Fig. 12.—Three longitudinal section of lower ephyra rudiment in fig. 9 showing form of endodermal groove which provides lining of manubrium (*a*) almost perradial, (*b*) about adradial, (*c*) near interradius and showing commencement of abaxial rod of cells.

ABBREVIATIONS.

C.S., connecting strand. *Cav.*, cavity of longitudinal muscle. *Col.*, columella. *Ect.*, ectoderm. *End.*, endoderm. *Ex.Ect.*, exumbral ectoderm. *Ex.End.*, exumbral endoderm. *G.*, gastral cavity. *G.F.*, gastral filaments. *G.P.*, gastral pouch. *Gr.*, groove of endoderm to form manubrium. *L.M.*, longitudinal muscle. *Mn.*, manubrium. *P.*, plug of endoderm cells to form interradial portion of manubrium. *P.P.*, peristomial pit. *R.*, rod of ectoderm cells to form peristomial pit of polyp. *R.C.*, remnant of exumbral end of columella after rupture. *R.L.M.*, degenerate longitudinal muscle. *S.*, split in ectoderm and in endodermal plug. *S.O.*, Septal ostium.